Appln No.: 09/601,644
Amendment Dated: November 21, 2005
Reply to Office Action of July 19, 2005

REMARKS/ARGUMENTS

This is in response to the Office Action mailed July 19, 2005 for the above-captioned application. Reconsideration and further examination are respectfully requested.

Applicants request an extension of time sufficient to make this paper timely and enclose the fee. The Commissioner is authorized to charge any additional fees associated with this amendment to Deposit Account No. 15-0610.

Applicants thank Examiner Shibuya for taking the time to meet with their attorney. This paper will serve as Applicants' summary of that interview.

During the interview the differences between using a toxin as a reporter for creation of molecules with binding moieties that bind to different types of cells, and how this is different from the references were discussed. The Examiner noted that the references both disclose testing for toxicity after mutation, and Applicants attorney pointed out that before testing for toxicity, the references had done binding assays to characterize the type of receptor binding element that was present. Thus, toxicity was not used to indicate or "report" that binding had occurred.

Following this explanation, the Examiner suggested that the art could be better overcome if the claims included specific limitations concerning selection or reporter function connected to toxicity. Further, the examiner suggested that a definition of the population of screening cells as cells that were not affected by the wild-type toxin in a parallel test, or cells that lacked the receptor for the wild type toxin should be considered.

In view of the Examiner's remarks, Applicants have amended claim 1 such that the the description of the screening step specifies observation of toxicity and selection based on the toxicity. In addition, claim 1 has been amended in step (A) to indicate that the heteromeric protein toxin is a ribosome inactivating protein, and to delete the word mutant from the phrase "wild-type cytotoxic mutant protein."

Applicants further note that claim 42 adds to claim 1 the limitation that the population of are "insensitive"to the wild type heteromeric protein toxin at the concentrations used for the screening." It should be appreciated that this does not mean that there is not some high concentration of the wild-type toxin that is toxic to the population of cells. Further, the term insensitive is not an absolute term, and encompasses the type of low decrease in cell viability that is observed in Figs. 4 and 6 for SKBr-3 and CAMA-1 in the presence of native toxin (open symbols).

Corresponding amendment have been made in withdrawn claims 32 and 37 to facilitate their recombination should claim 1 be found to be allowable following this amendment and the following remarks.

Claim 6 has been amended to correct a typographical error. Claim 8 has been canceled. Claim 43 has been added. Claim 43 is supported in the specification at Page 13, line 5.

Claims 1-7 and 9-16 are under examination as a result of the restriction requirement.

The Examiner states that the drawings are objected to because Fig. 1 appears to be missing from the drawings filed on August 4, 2000. Applicants direct the examiner's attention to the final page the claims in the electronic file copy, where Fig. 1 seems to have been incorrectly placed when the application was scanned in the PTO.

The Examiner rejected claims 1-16 and 42, second paragraph, as being indefinite, stating that "different receptor binding specificity" could be interpreted as meaning either "specific for a different receptor" or "receptor binding that is different (but where the receptor remains the same)." The Examiner states that because two meanings are possible, the claim is indefinite. Applicants respectfully disagree, because in this case, the claim language is intended to encompass both meanings. The specification refers to the first option presented by the Examiner, i.e., where the receptor is of a different type, *inter alia*, at Page 25, lines 26-28, and to the second type of variation, *inter alia*, on Page 20, line 30 - Page 21, lines 1, where binding to receptor homologs is noted.

The Examiner rejected claims 1-16 and 42 under 35 USC § 112, first paragraph, asserting a lack of written description. In support of this rejection, the Examiner argues that the claims are broadly directed to toxins, including toxins not specifically referenced in the application. Without conceding that this argument is well-founded, Applicants have amended claim 1 to refer to heteromeric protein toxins that are ribosome-inactivating proteins (RIP). RIPs are described on Page 6, lines 16-26, and include the Shiga and Shiga-like toxins used in the experiments a well as those listed in claim 9. These toxins form an art-recognized group, and a group that Applicants clearly recognized as part of their invention. Thus, a written description rejection based on this aspect of the claims is not appropriate.

The Examiner also argued that there was a lack of written description based on the identification of the cell type, because the two breast cancer cell lines are not representative of the genus of screening cells. Applicants respectfully traverse this rejection. The particular cell type used in the claimed method is not critical, provided the combination of toxin and cell type are such that a selection can be made based on an observed increase in toxicity, and the application fully reflects Applicants understanding of this scope of the invention. The

application refers to target cells generically (see for example, Page 7, lines 8-16, and screening for "constructs having specificity for unique cell targets (such as cancer cells)." (Page 8, lines 18-19) As stated on Page 25, "the results herein demonstrate the ease with which one can identify a collection of toxin mutants cytotoxic towards a relatively homogeneous cell population." Further, as stated on Pages 26-27, "a person skilled in the art will be appreciate that the method can be applied to other cells with the expectation that useful therapeutic and diagnostic molecules will be identified." Thus, the specification makes it clear that Applicants recognized and were therefore in possession of the scope of the invention as claimed. Thus, a written description rejection based on this aspect of the claims is not appropriate.

Finally, the Examiner asserts that written description is lacking because of the statement in the specification that "the B subunit variants may thus bind to a spectrum of molecular entities such as proteins, peptides, nucleic acids or even organic molecules rather than to sugars or glycolipids (such as CD77)" and the absence of disclosure of what types of receptor the mutated toxin in the examples has specificity for, or how it would be determined. As explained during the interview, however, an important benefit of the present invention is that it does not require any prior or subsequent knowledge of the specific nature of the receptor. The screening technique identifies, via observed toxicity, the mutation that works in combination with some receptor on the screening cells, and neither the nature of the receptor nor the nature of the mutation needs to be known. While it may be interesting to know the type of receptor a new toxin binds to, this is not a reason to say that there is no written description of the invention as claimed.

For these reasons, Applicants submit that the written description rejection should be withdrawn.

The Examiner also rejected claims 1-16 and 42 under 35 USC § 112, first paragraph, as lacking enablement. The focus of the rejection appears to be on the reference in the claims to a toxin with "different-receptor binding specificity." The Examiner also notes that the claims do not require that the screening cells lack the receptor to which the native toxin binds. Applicants respectfully traverse this rejection.

As discussed above, the present invention allows selection of toxins that are active against a cell type to a greater extent than the wild type toxin is active without any knowledge of the nature of receptor to which the toxin is binding. It follows from the observed difference in toxicity that the selected toxin has "different receptor-binding specificity than the wild type protein." The Examiner states that the art of ligand mutation and receptor specificity is unpredictable. If one were looking at consciously designing mutant ligands for binding to a certain receptor, this may be the case, but it is not relevant here. The point of Applicants' invention is to avoid this design phase (and any experimentation and unpredictability it may

entail) by using the cell for which a ligand is desired, and a toxin to identify when a successful receptor binding has occurred.

The Examiner also states that "methods for making mutant Shiga toxin and mutant Shiga-like toxin was known in the art at the time of filing, however, only a limited number of mutant Shiga toxin were known." This is not evidence of lack of enablement, but rather speaks to the difference between this invention and the prior art. As reflected in the present specification, multiple examples of toxins with different binding specificity and enhanced activity against SKB3-S or CAMA-1 were obtained from screening of a comparatively small number of mutant clones.

Furthermore, Applicants respectfully submit that the Examiner is applying a standard of enablement that looks far beyond whether a person skilled in the art could use the claimed method. The Examiner has offered no specific arguments that a person skilled in the art could not introduce mutations into a known toxin using the techniques described in the application, or test those mutations for toxicity using a screening cell line which is insensitive to the wild-type toxin, or recognize toxicity and choose to make more of the protein selected based on higher levels of toxicity, as compared to the wild type. This, being the case, Applicants submit that the Examiner has failed to present a *prima facie* case of lack of enablement of the claimed invention.

The Examiner has maintained the rejection of claims 1-3, 5, 9-13 and 16 as anticipated by Jackson et al. Based on earlier work that showed that the sequence of the B-subunit dictates cytotoxic specificity, Jackson generated and tested site-specific mutants of Shiga toxin and Shiga-like toxin to identify the domains associated with receptor binding, localization in E coli and recognition by monoclonal antibodies. Jackson prepared mutants of specific design (table 1), and tested the non-amber mutations for cytotoxicity (table 2). As characterized by Jackson (Page 655, Col. 2), only one of the mutations, D16H, D17H, had a "significant effect" on activity, and that effect was to eliminate the cytotoxicity for both HeLa and Vero cells and to eliminate receptor analog binding. Two mutant toxins showed a ten-fold decrease in cytotoxicity. In the case of the SLT-IIv B subunit mutations, the mutations either reduced the level of toxicity with respect to Vero cells or changed the distribution of the toxin between cell-associated and extracellular fractions. (Table 4).

Jackson does not perform a selection, as in step (D)(iv) of claim 1 based on the observation of toxicity. It is noted that in the Examiner's characterization of Jackson as compared to the claim method, no assertion that step (D) is performed is set forth in the rejection. To be anticipatory, however, the reference must disclose every limitation. Furthermore, Jackson does not report the occurrence of a mutant protein that has greater cytotoxicity than the native toxin.

The Examiner asserts that the burden is on Applicant to say that Jackson's method could not result in proteins that kill or inhibit cells to a greater extent than the wild-type. Applicants submit that this statement of burden is incorrect. First of all, there is nothing to say that the mutation method of Jackson could not be used for this purpose. However, the mutations chosen and reported by Jackson apparently did not result in any mutation of the type that is selected in the present invention. Thus, Jackson does not anticipate the claimed invention, nor does it suggest the screening method of the claimed invention as being a possibility.

The Examiner also rejected claims 1-3, 5, 9-13 and 16 as anticipated by Tyrell et al. As previously argued, Tyrell discloses making mutations of a toxin, but does not disclose the claimed method which is a method of finding a mutant toxin with different receptor binding specificity involving certain steps. Tyrell made his mutations and then tested for binding using a TLC assay (Table 1). He then took one of these mutants that showed a binding change, selected based on the binding assay, and compared its toxicity with that of the wild type. Thus, Tyrell does not teach the claimed method in which the selection process is done based on the observation of toxicity.

The Examiner rejected the other dependent claims as obvious over combinations of Jackson or Tyrell with secondary references. Applicants again submit that these references do not overcome the fundamental deficiencies of the Jackson and Tyrell references, and these rejection should therefore be withdrawn. Nothing in Tyrell or Jackson teaches or suggests the claimed method in which the selection of a protein or pool of proteins having altered receptor-binding behavior is made based upon an observation of increased toxicity.

It is further noted that new claim 43 which recites random mutagenesis is not anticipated by or obvious over Jackson or Tyrell, both of which made mutations at specific and selected sites in the sequence for specific purposes.

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For these reasons, this application is now considered to be in condition for allowance and such action is earnestly solicited. Upon allowance of claim 1, Applicants submit that claims 18, 27, 32, 37 and the claims dependent thereon are properly recombined for allowance in a single application.

Respectfully submitted,

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Enclosures: Request for extension of time
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